

## Bennett, John

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**From:** Fout, Shay  
**Sent:** Thursday, January 08, 2015 10:49 AM  
**To:** 'Li, Charles'  
**Subject:** RE: Water Cluster Project

Yes, go ahead and run the QC on EV, GIB and GII using the armored RNA standard. Please run a QC on the HGV as well, but please check to make certain I am sending you sufficient reagents for the QC tests. I was originally thinking that we would run the QC here using the standard curve as the positive QC and no template controls as the negative QC and only included enough reagents to make the plates; however, it certainly would be best for you to make certain all mixes are good before making the plates.

G. Shay Fout, Ph.D.  
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**From:** Li, Charles [<mailto:cli@EMSL.com>]  
**Sent:** Thursday, January 08, 2015 10:34 AM  
**To:** Fout, Shay  
**Subject:** RE: Water Cluster Project

Hi, Shay,

Thanks. Yes, all primers and probes are in the supplies for making the kits. Shall I run QC tests for EV, GIB and GII using Armored RNA? I understand HGV has been used as QC in the RT-qPCR.

Charlie



**Charles Li, Ph.D.** | *Director of PCR and DNA Analysis Laboratory*

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**From:** Fout, Shay [<mailto:Fout.Shay@epa.gov>]  
**Sent:** Thursday, January 08, 2015 10:31 AM  
**To:** Li, Charles  
**Subject:** RE: Water Cluster Project

Hi Charlie,  
I'm sending all the primers and probes and can send a vial of the armored RNA in addition. The armored RNA has a titer of  $2.5 \times 10^8$  genomic copies per mL in a volume of about 0.25 mL.  
Shay

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**From:** Li, Charles [<mailto:cli@EMSL.com>]  
**Sent:** Thursday, January 08, 2015 9:03 AM  
**To:** Fout, Shay  
**Subject:** RE: Water Cluster Project

Hi, Shay,

Will you provide QC reagents for the RT-qPCR kits? I need to run QC on Master Mixes, so I need qPCR primers and probes, and viral RNA or armored RNA as controls, the master mixes must show detections for viral RNAs, no contamination before I put the mixes into qPCR plates.

Thanks

Charlie



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**From:** Fout, Shay [<mailto:Fout.Shay@epa.gov>]  
**Sent:** Wednesday, January 07, 2015 2:01 PM

**To:** Li, Charles  
**Subject:** RE: Water Cluster Project

Hi Charlie,  
I still don't have the dry ice, so it will have to wait until I return on the 20<sup>th</sup>. I'm at work this week through Friday. Thanks,  
Shay

G. Shay Fout, Ph.D.  
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**From:** Li, Charles [<mailto:cli@EMSL.com>]  
**Sent:** Wednesday, January 07, 2015 1:35 PM  
**To:** Fout, Shay  
**Subject:** RE: Water Cluster Project

Hi, Shay,

I have StepOnePlus and the 96 well plates. You do not need to send. I can wait when you return. I am going to send the protocol-my version to you soon, so you can review it.

Thanks

Charlie



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**From:** Fout, Shay [<mailto:Fout.Shay@epa.gov>]  
**Sent:** Wednesday, January 07, 2015 1:25 PM  
**To:** Li, Charles  
**Subject:** RE: Water Cluster Project

Charlie,

We will use generic 96 well plates for the RT step and the One Step Plus System for the PCR step. This instrument uses the MicroAmp Fast Optical 96-Well Reaction Plates. I can send you some of each tomorrow. Attached is the latest version of the SOW which reflects some change from the last version I sent. It also shows what I will be sending you in terms of reagents. I may not get the reagents sent out today, as my dry ice that I was saving has evaporated. One of my colleagues got some packages in and there may be enough to use, but I have to leave at 3 today for a doctor's appointment, so there may not be enough time.

Shay

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---

**From:** Li, Charles [<mailto:cli@EMSL.com>]  
**Sent:** Wednesday, January 07, 2015 11:56 AM  
**To:** Fout, Shay  
**Subject:** RE: Water Cluster Project

Hi, Shay,

Which type of 96-well plates do you use in your lab? I know they are instrument specific. I will send you a working protocol for approval soon.

Thanks

Charlie



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**From:** Fout, Shay [<mailto:Fout.Shay@epa.gov>]  
**Sent:** Tuesday, January 06, 2015 1:59 PM  
**To:** Li, Charles  
**Subject:** RE: Water Cluster Project

Hi Charlie,

I had hoped to send you the reagents yesterday, but have to get a paper resubmitted this week. The first author has been out of the country and did not have the ability to do the edits; the journal said we have to get it back or we would have to resubmit. I had to take over the edits and am almost finished, but won't have time to get everything together today. Will you be in on Friday? If so, I will try to send the package tomorrow. If not, I'm out of the office all next week, so it would have to wait until 20<sup>th</sup> before I could send it.

Happy New Year.  
Shay

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**From:** Li, Charles [<mailto:cli@EMSL.com>]  
**Sent:** Tuesday, December 09, 2014 9:09 AM  
**To:** Fout, Shay  
**Subject:** RE: Water Cluster Project

Hi, Shay,

Winter season is good time for me to work with you on research projects. I will work on the kits when the reagents arrive.

Thanks & Happy Holiday to You!

Charlie



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**From:** Fout, Shay [<mailto:Fout.Shay@epa.gov>]  
**Sent:** Tuesday, December 09, 2014 7:53 AM  
**To:** Li, Charles  
**Subject:** RE: Water Cluster Project

Hi Charlie,

I apologize for the delay in follow-up to our last email exchange. The last couple of months have been hectic, especially early in the week. I meant to send you the reagents before now, but with the holidays, I think it would be best to wait until after the 1<sup>st</sup>, thus I send them by overnight delivery on 1/5/15. We have collected most of the samples needed for the kit testing and will finish the collection next week, and then do extractions on 1/7. We can then do the kit testing at your convenience in late January or during February. I will contact you when I send the reagents and get your time frame for the kit preparation.

Have a Happy Holiday season,  
Shay

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**From:** Li, Charles [<mailto:cli@EMSL.com>]  
**Sent:** Thursday, October 23, 2014 8:51 AM  
**To:** Fout, Shay  
**Subject:** RE: Water Cluster Project

Hi, Shay,

That is fine. Thanks

Charlie



**Charles Li, Ph.D. | Director of PCR and DNA Analysis Laboratory**

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**From:** Fout, Shay [<mailto:Fout.Shay@epa.gov>]  
**Sent:** Thursday, October 23, 2014 8:45 AM  
**To:** Li, Charles  
**Subject:** RE: Water Cluster Project

Hi Charlie,

Everyone in my Branch has been too tied up with other projects to get the samples completed over the last two months, but it looks like we will be able to complete them during November. December will probably be too busy to do the analyses, so I'm thinking about targeting the method and kit analyses for January. Will that be workable for your group?

It also took longer than I expected to get all the reagents, but I have received them and will be sending them to you either next week or the week after.

Thanks and best regards,  
Shay

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**From:** Li, Charles [<mailto:cli@EMSL.com>]  
**Sent:** Wednesday, August 27, 2014 9:32 AM  
**To:** Fout, Shay  
**Subject:** RE: Water Cluster Project

Hi, Shay,

There is no problem from my end. I will make your project as top priority and I have analysts working with me on routine samples.

Thanks

Charlie



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**From:** Fout, Shay [<mailto:Fout.Shay@epa.gov>]  
**Sent:** Wednesday, August 27, 2014 9:17 AM  
**To:** Li, Charles  
**Subject:** RE: Water Cluster Project

Hi, Dr. Li,

I want to give you an update of our status. I have ordered all the supplies needed and will send you portions to you next week, assuming everything arrives.

The schedule for collecting the samples is slipping from what I had originally anticipated. We have collected and processed six reagent grade water samples (seeded with primary effluent), but have decided that we need to run a pretest on a couple of them using the new reagents before collecting the remaining sample sets. We want to make certain that there is enough virus in the amount of seed added to give decent Ct values. In addition, the staff are overwhelmed with other project deadlines, so it will probably be the end of October or early November before we will be ready for the complete assays. This will delay your preparation of the test kits until about that time. Please let me know if there will be any problems with the delay.

Thanks and best regards,  
Shay

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---

**From:** Li, Charles [<mailto:cli@EMSL.com>]  
**Sent:** Tuesday, July 15, 2014 4:43 PM  
**To:** Fout, Shay  
**Subject:** RE: Water Cluster Project

Hi, Dr. Fout,

I will work on them directly without delays, I think we are better to use in weeks, I will try to ship to you in the same week or next week.

Charlie



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**From:** Fout, Shay [<mailto:Fout.Shay@epa.gov>]  
**Sent:** Tuesday, July 15, 2014 1:33 PM  
**To:** Li, Charles  
**Subject:** RE: Water Cluster Project



Hi Dr. Li,

I failed to mention that it will take time to collect and process all the samples mentioned in the statement of work. I have a meeting with my EPA team tomorrow, so should have a better feel for how long, but it will probably take a couple of months to get all of them ready. What is your experience with shelf life of the plates you prepare? Can we go ahead and have you prepare the plates as soon as we get funding and expect them to be fine if there is a several month delay before they are used?

Best regards,  
Shay

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---

**From:** Li, Charles [<mailto:cli@EMSL.com>]

**Sent:** Tuesday, July 15, 2014 12:29 PM

**To:** Fout, Shay; Dombro, Danielle

**Cc:** Dobranic, Jason; Grimm, Ann

**Subject:** RE: Water Cluster Project

Hi, Dr. Fout,

We consider EPA research project as our top priority work, we have adequate resources to cover the work listed. We will meet your completion time requirement.

Thanks

Charlie



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**From:** Fout, Shay [<mailto:Fout.Shay@epa.gov>]

**Sent:** Tuesday, July 15, 2014 11:28 AM

**To:** Li, Charles; Dombro, Danielle

**Cc:** Dobranic, Jason; Grimm, Ann  
**Subject:** RE: Water Cluster Project

Hi Dr. Li,

Please find attached the signed UBMTA with the complete Statement of Work. The funds that will be used to purchase the necessary reagents have to be transferred from one EPA account to another. I'm not certain how long that will take, but will let you know when I can start ordering supplies. Is there a particular time over the next 1-3 months when you would like to start your part of the cooperative effort?

I look forward to working with you.

Shay

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---

**From:** Li, Charles [<mailto:cli@EMSL.com>]  
**Sent:** Tuesday, July 15, 2014 10:39 AM  
**To:** Fout, Shay  
**Subject:** RE: Water Cluster Project

Hi, Dr. Fout,

I read the SOP and I do not see any problem to follow it and make the plates and reagents mix for you.

Let me know what you need from me.

Thanks

Charlie



**Charles Li, Ph.D.** | *Director of PCR and DNA Analysis Laboratory*

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